DELETERIOUS EFFECTS OF SIMULATED SPACEFLIGHT ON BONE AND MICROVASCULATURE IN ADULT MICE

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Long-term spaceflight leads to extensive changes in the musculoskeletal system attributable, in part, to unloading during microgravity exposure. Additionally, irradiation at doses similar to that of a solar flare or a round-trip sojourn to Mars may cause significant depletion of stem/progenitor cell pools throughout the body as well as inflammation associated with prompt skeletal-tissue degradation. Previously, we demonstrated that irradiation leads to rapid bone loss, which can be mitigated in the short term by injection of a potent antioxidant (α-lipoic acid). Furthermore, simulated weightlessness in adult mice adversely affects skeletal responses to low linear energy transfer (LET) radiation (^{137}Cs). Here, we hypothesized that simulated weightlessness exacerbates the adverse effects of simulated space radiation (including both protons and ^{56}Fe ions) by adversely affecting skeletal structure and functions as well as associated vasculature. Furthermore, we hypothesized that an antioxidant cocktail, which has been shown to be protective in other tissues (Kennedy *et al*., Radiat Res. 2011), mitigates space radiation-induced bone loss.

Male, 16-week old, C57BL6/J mice were used to test our hypotheses. We tested radioprotective properties of a diet supplemented with an antioxidant cocktail (Ascorbic acid 142.8 mg/kg, N-Acetyl Cystine 171.4 mg/kg, L-Selenomethionine 0.06 mg/kg, α-lipoic acid 85.7 mg/kg, vitamin E 71.4 mg/kg) and its ability to mitigate simulated space radiation-induced bone loss and inhibition of osteoblastogenesis. Mice were pre-acclimating mice to the diet for one week before radiation exposure. Cancellous bone microarchitecture in the tibial metaphysis was quantified with microcomputed tomography (6.7 μm resolution). Radiation exposure (^{56}Fe alone at 100cGy or sequential ^{1}H/^{56}Fe/^{1}H at 25/50/25cGy doses) caused bone loss and deterioration of cancellous microarchitecture in the tibial metaphysis. Our preliminary results show dietary supplementation with the antioxidant cocktail did not prevent radiation-induced loss of cancellous bone volume or changes in other microarchitectural parameters 11 days after radiation exposure. At the cellular level, total body irradiation with ^{56}Fe decreased colony formation in *ex vivo* osteoblastogenic cultures of bone marrow cells extracted from the animals, and the antioxidant diet did not appear to mitigate the deleterious effects of radiation on osteoblast colony formation 17 days post irradiation. Further analyses are in progress.

To determine if simulated space radiation and weightlessness cause changes in both bone and vascular function, we subjected mice to hindlimb unloading for 14 days. Three days after initiating hindlimb unloading, mice were exposed to ^{56}Fe ion (600 MeV) at 100cGy or sham-irradiated. Exposure to radiation alone caused significant decrements in percent bone volume, trabecular number and structural model index, but not trabecular thickness. Trabecular thickness was significantly reduced by hindlimb unloading alone, whereas other variables were unchanged. Unloading and radiation combined had no additional significant effects on cancellous bone microarchitecture. We also evaluated the endothelium dependent and independent vasodilator responses of gastrocnemius feed arteries that were isolated, cannulated and pressurized for *in vitro* study. In all treatment groups, endothelium-dependent and independent vasodilation responses were impaired relative to controls. The combined effects of hindlimb unloading and irradiation further depressed endothelium-dependent vasodilation. Peak endothelium-dependent vasodilation was positively correlated (R^2=0.61, P<XXX) with cancellous bone volume to total volume fraction.

Taken together, these results support the theory of a bone-vascular coupling mechanism that contributes to altered skeletal remodeling due to simulated spaceflight, and implicate an important role for the regulation of endothelial signaling pathways by both ionizing radiation and musculoskeletal disuse. Further development is needed to determine the value of dietary manipulation with the goal of mitigating spaceflight-induced bone loss.

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